Dendritic Cell Membrane Protein FIRE

FIELD OF THE INVENTION

The present invention relates to a protein (designated FIRE) which is preferentially expressed in dendritic cells stimulatory to T cells, macrophages and their precursors and to nucleic acid sequences encoding this protein. The invention also relates to uses of the protein and nucleic acids.

BACKGROUND OF THE INVENTION

Dendritic cells (DC) are antigen presenting leukocytes which play a critical role in the initiation of immune responses. To stimulate naive T lymphocytes, which is an essential step in generating the immunological memory required for effective vaccination, it is crucial for antigen to be presented by DC. Over the last 8 years techniques have been developed to purify DC populations and lineages from mouse lymphoid organs. This DC purification protocol involves density centrifugation, depletion of contaminating cells with a monoclonal antibody cocktail and magnetic beads, and finally Fluorescent Activated Cell Sorting. Using an original version of this purification protocol, two DC populations in mouse spleen were identified, which are defined by their expression of two cell surface proteins: a lymphoid-lineage related $CD8^+Mac\text{-}1^-$ DC and a myeloid-lineage related $CD8^-Mac\text{-}1^+$ DC. These two populations differ in their interactions with T lymphocytes. Although the two DC populations displayed equivalent ability to stimulate T cells into cell cycle, they differed in their ability to induce the production of cytokines such as IL-2 and IL-3, which are critical for the induction of an effective immune response (1-5). The myeloid related $CD8^-Mac\text{-}1^+$DC are much more efficient in cytokine induction than the lymphoid related $CD8^+Mac\text{-}1^-$ DC. Other differences between the DC are the levels of cytokines they themselves produce (such as IL-12) which, potentially, could regulate the nature as well as the quality of cytokines they induce in the activated T cells. The molecular mechanisms which underpin these differences in interactions between the two DC populations and T lymphocytes are unknown. Presumably myeloid DC differentially express molecules which enable them to stimulate T lymphocytes to produce certain cytokines more efficiently than do lymphoid DC, or alternatively lymphoid

DC differentially express molecules which inhibit the stimulation of T lymphocytes. To address this question the present inventors compared gene expression in the two DC populations using the technique of Representational Difference Analyses (RDA)(6).

Briefly, RDA identifies differential gene expression between two given cell types by using successive rounds of a combination of PCR and subtractive hybridisation which generates DNA fragments of putatively differentially expressed genes (6).

A full length clone encoding a novel mouse gene was obtained using conventional molecular biological techniques, involving the RDA generated fragments. This sequence was designated "FIRE" and encodes a 681 amino acid protein. Analyses of the FIRE sequence shows that it is a novel member of the recently described EGF/TM7 superfamily (7). The mouse sequence was then used to isolate the equivalent human FIRE sequence.

SUMMARY OF THE INVENTION

Accordingly, in a first aspect the present invention consists in an isolated polypeptide, the polypeptide comprising:-

(i) an amino acid sequence as set out in SEQ ID NO:1, or

(ii) an amino acid sequence having at least 50% identity to the amino acid sequence set out in SEQ ID NO:1, or

(iii) a functional fragment of (i) or (ii).

In a preferred embodiment of the first aspect, the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:1.

In a second aspect, the present isolated polypeptide, the polypeptide comprising:-

(i) an amino acid sequence as set out in SEQ ID NO:2, or

(ii) an amino acid sequence having at least 50% identity to the amino acid sequence set out in SEQ ID NO:2, or

(iii) a functional fragment of (i) or (ii).

In a preferred embodiment of the second aspect, the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:2.

In a further preferred embodiment of the first and second aspects, the polypeptide is expressed on dendritic cells.

The term "functional fragment" as used herein is intended to cover fragments of the polypeptide which retain at least 10% of the biological activity of the complete polypeptide. In particular this term is used to encompass fragments which show immunological cross-reactivity with the entire polypeptide, eg ligands which interact with the fragment also interact with the complete polypeptide.

In a third aspect the present invention consists in an isolated ligand, the ligand being directed against the polypeptide of the first aspect of the present invention.

The ligand may be an inorganic or organic molecule. In one preferred embodiment the ligand is an antibody or the binding portion thereof.

In a fourth aspect the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule encoding a polypeptide of the first or second aspects.

In a fifth aspect, the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule comprising:-

(i) a sequence as set out in SEQ ID NO:3, or

(ii) a sequence having at least 60% identity to the sequence set out in SEQ ID NO:3, or

(iii) a sequence which hybridises to the sequence set out in SEQ ID NO:3 under stringent conditions.

In a preferred embodiment of the fifth aspect, the nucleic acid molecule comprises a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:3.

In a further preferred embodiment of the fifth aspect, the isolated nucleic acid molecule has at least 95% identity to the nucleotide sequence shown in SEQ ID NO:3, preferably within the region from nucleotide 218 to 2260.

In a sixth aspect the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule comprising:-

(i) a sequence as set out in SEQ ID NO:4, or

(ii) a sequence having at least 60% identity to the sequence set out in SEQ ID NO:4, or

(iii) a sequence which hybridises to the sequence set out in SEQ ID NO:4 under stringent conditions.

In a preferred embodiment of the sixth aspect, the nucleic acid molecule comprises a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:4.

In a further preferred embodiment of the sixth aspect, the isolated nucleic acid molecule has at least 95% identity to the nucleotide sequence shown in SEQ ID NO:4 within the region from nucleotide 1 to 1903.

In a seventh aspect the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule encoding the binding region of a ligand of the third aspect.

In a further preferred embodiment of the present invention, the nucleic acid molecules of the present invention are preferably less than 5000 nucleotides, however, they may be less than 1000 or 500 nucleotides in length. Preferably, the nucleic acid molecules of the present invention are at least 18 nucleotides in length.

An "isolated" polypeptide or ligand refers to a polypeptide or ligand that has been substantially isolated from other proteins, lipids, nucleic acids and other contaminants.

An "isolated" nucleic acid molecule refers to a nucleic acid molecule that is one or both of the following: not immediately contiguous with both of the coding sequences with which it is immediately contiguous in the naturally occurring genome of the organism from which the nucleic acid is derived; or which is substantially free of other nucleic acid, proteins, lipids, and other contaminants.

In an eighth aspect the present invention consists in a composition for use in raising or lowering an immune response in a subject, the composition comprising a ligand of the third aspect of the present invention and an antigen and optionally a carrier and/or adjuvant.

In a preferred embodiment the antigen is associated with the ligand. The antigen may be associated with the ligand by any suitable means known in the art. Suitable methods for associating the ligand and antigen are described, for example, in Cox, J. and Coulter, A.R. (1999) Biodrugs 12:439-453.

In a further preferred embodiment, the antigen is conjugated to the ligand.

It will be appreciated by those skilled in the art that in the context of the eighth aspect, any antigen of interest may be used in the composition. For example, the antigen may be derived from an infectious pathogen or from a tumour cell.

In a ninth aspect the present invention consists in a composition for use in raising or lowering an immune response in a subject, the composition comprising a nucleic acid molecule and a carrier, the nucleic acid molecule comprising a first sequence encoding a ligand of the third aspect of the present invention and a second sequence encoding an antigen.

In a tenth aspect the present invention consists in a method of screening compounds for immunological regulatory activity, the method comprising reacting the compound with the polypeptide or peptide of the first aspect of the invention and measuring interaction between the compound and the polypeptide or peptide.

As will be appreciated by those skilled in the art, the polypeptides, peptides and nucleic acid molecules of the present invention provide useful markers of subgroups of dendritic cells and antigen presenting cells (such as macrophages). They also provide useful markers of dendritic cell precursors.

The nucleic acid molecules of the present invention may also be used as tools to analyse the properties and functions of the FIRE gene/protein. For example, the nucleic acid molecules may be used to generate animal models, preferably mouse models, wherein the animals lack functional FIRE genes. Alternatively, the nucleic acid molecules may be introduced and expressed in cells in which the FIRE gene is not normally expressed.

It will also be appreciated that the nucleic acid molecules of the present invention may be used to isolate regulatory regions (such as the promoter region) of the FIRE gene. Such regulatory regions may be used to selectively express exogenous genes in dendritic or antigen presenting cells.

The ligands of the present invention may be used to isolate dendritic cells, dendritic cell precursors, or other antigen presenting cells, from biological samples (eg. from blood). Accordingly, these ligands may be used in various immunisation processes. For example, the cells which are isolated from a patient through use of these ligands may be grown *in vitro*, exposed to one or more antigens, and then introduced back into the patient.

The ligands of the present invention may also be used to modulate immune responses by interfering with the function, migration or maturation

of dendritic or antigen presenting cells. Ligands which act as agonists or antagonists may be useful in the modulation of immune responses. For example, ligands of the present invention may be administered to patients under conditions such that the ligands bind to and interfere with the function of myeloid dendritic cells, with the result that antigen processing is undertaken by lymphoid dendritic cells. This may lead to immune suppression and anergy, a desirable outcome in the treatment of allergies and autoimmune disorders.

The ligands of the present invention may also be used to target molecules, such as vaccine components, to dendritic or antigen presenting cells. Suitable methods for targeting will be known to those skilled in the art. Non-limiting examples of suitable taragetting methods are described in WO 98/44129.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: FIRE-FLAG is presented on the surface of stably transfected CHO cells as detected by immunofluorescent staining and flow cytometry using anti-FLAG mAb (IC7) and anti-mouse-PE. The filled histograph represents CHO cells transfected with the neomycin resistance gene only, stained with anti-FLAG mAb whilst the hollow histograph are CHO-FIRE-FLAG cells staining positive for the FLAG epitope.

Figure 2 shows the full length cDNA sequence of mouse FIRE and the translated protein sequence.

Figure 3 shows a comparison of the protein sequence of FIRE with members of the EGF/TM7 superfamily including human Emr1 (SEQ ID NO:5); mouse EMR1 (SEQ ID NO:6) and human CD97 (SEQ ID NO:7).

Figure 4: Immunoflurescent staining of FIRE on spenic DC, splenic macrophages and blood mononuclear cells. A) DC were extracted and purified from spleens of C57BL/6 mice and stained with anti-CD11c, anti-CD4, anti-CD8 and anti-FIRE. The cells that were gated on expressed high levels of CD11c and high forward scatter. Dead cells staining with PI were gated out using the FL5 channel. DC that failed to express CD4 and CD8 expressed the highest level of FIRE. CD4+ and CD8+ DC express lower levels of FIRE. Smooth line denotes background control staining and dotted line indicates staining with FIRE. B) Macrophages were extracted and purified from spleens of C57BL/6 mice and stained with anti-CD11b, anti-F4/80, and anti-FIRE. C) Macrophages, defined as the population of cells that express high levels of both F4/80 and CD11b, also express FIRE. Smooth line denotes background control staining and dotted line indicates staining with FIRE. D) Blood mononuclear cells were obtained from C57BL/6 mice by centrifugation over a gradient (lympholyte M), then depleted of cells expressing CD3, GR-1, TER119, Thy1.1 and B220. A large proportion of the remaining blood mononuclear cells expressed FIRE.

Figure 5: *In vitro* culture of FIRE+ blood mononuclear cells gives rise to a high proportion of CD11c positive cells. Peripheral blood mononuclear cells were isolated as described above. Cells were stained with anti-FIRE mAb then sorted on the basis of FIRE expression. Both FIRE+ (A and B) and FIRE- (C and D) cells were incubated in medium alone (A and C) or medium plus IL-4, FL3L, TNF-α, and GM-CSF (B and D).

Figure 6 shows the cDNA sequence of human FIRE and the translated protein sequence.

DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting Examples.

General Molecular Biology

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

Protein Variants

Amino acid sequence variants can be prepared by introducing appropriate nucleotide changes into DNA, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired characteristics. The amino acid changes also may alter post-translational processes such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, altering the intra-cellular location by inserting, deleting or otherwise affecting the transmembrane sequences of the native protein, or modifying its susceptibility to proteolytic cleavage.

In designing amino acid sequence variants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and

then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of other ligands adjacent to the located site.

A useful method for identification of residues or regions for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (*Science* (1989) 244: 1081-1085). Here, a residue or group of target residues are identified (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimise the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants; the location of the mutation site and the nature of the mutation. These may represent naturally occurring alleles or predetermined mutant forms made by mutating the DNA either to arrive at an allele or a variant not found in nature. In general, the location and nature of the mutation chosen will depend upon the characteristic to be modified.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Amino acid sequence insertions include amino and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Other insertional variants include the fusion of the N- or C-terminus of the proteins to an immunogenic polypeptide e.g. bacterial polypeptides such as betalactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, bovine serum albumin, and chemotactic polypeptides. C-terminal fusions with proteins having a

long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, are included.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro	pro
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe norleucine	leu
Leu (L)	norleucine, ile; val; met; ala; phe	ile

Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile;	leu
Phe (F)	leu; val; ile; ala	leu
Pro (P)	gly	gly
Ser (S)	thr	thr
Thr (T	ser	ser
Trp (W)	tyr	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Mutants, Variants and Homology - Proteins

Mutant polypeptides will possess one or more mutations which are deletions, insertions, or substitutions of amino acid residues. Mutants can be either naturally occurring (that is to say, purified or isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the encoding DNA). It is thus apparent that polypeptides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Protein sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the protein will be the equivalent protein which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the protein. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A protein at least 50% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 70% or 80%

and more preferably at least 90% identical to the protein of the present invention. This will generally be over a region of at least 20, preferably at least 30, contiguous amino acids.

Mutants, Variants and Homology - Nucleic Acids

Mutant polynucleotides will possess one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the DNA). It is thus apparent that polynucleotides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Nucleotide sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the polynucleotide will be the equivalent polynucleotide which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the polynucleotide. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A polynucleotide at least 60% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 80% or 90% and more preferably at least 95% identical to the polynucleotide of the present invention. This will generally be over a region of at least 60, preferably at least 90, contiguous nucleotide residues.

Antibody Production

Antibodies, either polyclonal or monoclonal, which are specific for a protein of the present invention can be produced by a person skilled in the

art using standard techniques such as, but not limited to, those described by Harlow et al. Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press (1988), and D. Catty (editor), Antibodies: A Practical Approach, IRL Press (1988).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a protein. For the production of polyclonal antibodies, a number of host animals are acceptable for the generation of antibodies by immunization with one or more injections of a polypeptide preparation, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response in the host animal, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminium hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole lympet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

A monoclonal antibody to an epitope of a protein may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256, 493-497), and the more recent human B-cell hybridoma technique (Kesber *et al.* 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole *et al.* 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" by splicing the genes from an antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity may be used (Morrison *et al.* 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger *et al.* 1984 Nature 312:604-608; Takeda *et al.* 1985 Nature 31:452-454). Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce 4-specific single chain antibodies.

Recombinant human or humanized versions of monoclonal antibodies are a preferred embodiment for human therapeutic applications. Humanized antibodies may be prepared according to procedures in the literature (e.g. Jones *et al.* 1986, Nature 321:522-25; Reichman *et al.* 1988, Nature

332:323-27; Verhoeyen *et al.* 1988, Science 239:1534-36). The recently described "gene conversion mutagenesis" strategy for the production of humanized monoclonal antibody may also be employed in the production of humanized antibodies (Carter *et al.* 1992 Proc. Natl. Acad. Sci. U.S.A. 89:4285-89). Alternatively, techniques for generating the recombinant phage library of random combinations of heavy and light regions may be used to prepare recombinant antibodies (e.g. Huse *et al.* 1989 Science 246:1275-81).

Antibody fragments which contain the idiotype of the molecule such as Fv $F(ab^1)$ and $F(ab^2)$ may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab) E2 fragment which can be produced by pepsin digestion of the intact antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Alternatively, Fab expression libraries may be constructed (Huse *et al.* 1989, Science 246:1275-1281) to allow rapid and easy cloning of a monoclonal Fab fragment with the desired specificity to a protein.

Adjuvants and Carriers

Pharmaceutically acceptable carriers or diluents include those used in compositions suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. They are non-toxic to recipients at the dosages and concentrations employed. Representative examples of pharmaceutically acceptable carriers or diluents include, but are not limited to water, isotonic solutions which are preferably buffered at a physiological pH (such as phosphate-buffered saline or Tris-buffered saline) and can also contain one or more of, mannitol, lactose, trehalose, dextrose, glycerol, ethanol or polypeptides (such as human serum albumin). The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

As mentioned above the composition may include an adjuvant. As will be understood an "adjuvant" means a composition comprised of one or more substances that enhances the immunogenicity and efficacy of a vaccine composition. Non-limiting examples of suitable adjuvants include squalane

and squalene (or other oils of animal origin); block copolymers; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); interleukins such as interleukin 2 and interleukin 12; monokines such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; liposomes: ISCOM adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as murarmyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran or with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (e.g. U.S. Pat. No. 5,047,238); vaccinia or animal poxvirus proteins; sub-viral particle adjuvants such as cholera toxin, or mixtures thereof.

Gene/DNA Isolation

The DNA encoding a protein may be obtained from any cDNA library prepared from tissue believed to express the gene mRNA and to express it at a detectable level. DNA can also be obtained from a genomic library.

Libraries are screened with probes or analytical tools designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind the protein; oligonucleotides of about 20-80 bases in length that encode known or suspected portions of cDNA from the same or different species; and/or complementary or homologous cDNAs or fragments thereof that encode the same or a hybridizing gene. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or hybridizing DNA including expressed sequence tags and the like; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*

An alternative means to isolate a gene encoding the protein of interest is to use polymerase chain reaction (PCR) methodology as described in

section 14 of Sambrook *et al.* This method requires the use of oligonucleotide probes that will hybridize to the gene.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The actual nucleotide sequence(s) is usually based on conserved or highly homologous nucleotide sequences or regions of the gene. The oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is known. The oligonucleotide must be labelled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labelling is to use (α-^{32}P)- dATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labelling.

DNA encompassing all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al.*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

Another alternative method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Fingels *et al.* (*Agnew Chem. Int. Ed. Engl. 28:* 716-734, 1989). These methods include triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

Nucleic acid hybridisation

The polynucleotide sequence of the present invention may hybridise to the sequence set out in SEQ ID NO:3 or SEQ NO:4 under high stringency. As used herein, stringent conditions are those that (i) employ low ionic strength and high temperature for washing after hybridization, for example, 0.1 x SSC

and 0.1% (w/v) SDS at 50°C; (ii) employ during hybridization conditions such that the hybridization temperature is 25°C lower than the duplex melting temperature of the hybridizing polynucleotides, for example 1.5 x SSPE, 10% (w/v) polyethylene glycol 6000, 7% (w/v) SDS, 0.25 mg/ml fragmented herring sperm DNA at 65°C; or (iii) for example, 0.5M sodium phosphate, pH 7.2, 5mM EDTA, 7% (w/v) SDS and 0.5% (w/v) BLOTTO at 70°C; or (iv) employ during hybridization a denaturing agent such as formamide, for example, 50% (v/v) formamide with 5 x SSC, 50mM sodium phosphate (pH 6.5) and 5 x Denhardt's solution at 42°C; or (v) employ, for example, 50% (v/v) formamide, 5 x SSC, 50mM sodium phosphate (pH 6.8), 0.1% (w/v) sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50μg/ml) and 10% dextran sulphate at 42°C.

EXPERIMENTAL DETAILS

MATERIALS AND METHODS

1. Isolation of Dendritic cells (DC)

The procedure for the isolation of DC subpopulations has been described elsewhere in detail (Vremec *et al*, (1992). J. Exp. Med. 176: 47-58; Kronin *et al*. (1996). J. Immunol, 157; 3819). Briefly, spleens were digested with collagenase (1mg/ml; Worthington type II) and DNAase at room temperature for 20 min, followed by EDTA treatment for 5 min to disrupt DC-T cell complexes. Remaining procedures were conducted at 4°C. Low density cells were enriched by centrifugation for 10 min in Nycodenz medium (1.077 g/cm^3 mouse osmolarity). The low density cells were incubated with a mixture of mAb consisting of: anti-CD3, KT3-1.1; anti-CD4, GK1.5; anti-Th1.2, 30-H12; anti-Gr-1, RB68C5; anti-F4/80, anti-B220, RA36B2; and anti-erythrocytes, TER119. All the mAb were used at pre-titrated levels. Antibody coated cells were depleted with anti-rat IgG-conjugated magnetic beads, used at 5:1 bead-to-cell-ratio. The remaining cells were stained with fluorochrome-conjugated anti-CD11c and anti-CD8α mAb and propidium iodide (to label and exclude dead cells). Populations of > 95% pure viable CD11c$^+$ CD8α^+ and CD11c$^+$ CD8α^- DC were isolated by sorting on MoFlow (Cytomation Inc.). Cells were snap frozen and stored at -70°C until used to extract RNA.

2. RDA (Representational Difference Analysis)

RNA was extracted using QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech) and cDNA was synthesised (cDNA Synthesis Kit, Boehringer Mannheim Biochemica) according to the manufacturer's instruction. The cDNA RDA method was essentially as described by Hubank and Schatz (Nuc. Acid. Res. 22: 5640-5648, 1994). Minor alternations to this protocol include the amount of starting RNA. Due to the scarcity of the two DC populations, a total of 5×10^5 $CD8^-Mac\text{-}1^+$ DC and 1.8×10^8 $CD8^+Mac\text{-}1^-$ DC were used to extract mRNA. The synthesised double stranded cDNA was then digested with *DpnII* and purified by phenol extraction and ethanol precipitation in the presence of 2 µg glycogen. Digested cDNA was annealed with R-Bgl-24 and R-Bgl-12 and ligated with T4 DNA Ligase (1200 units) at 14°C for 12-16 h. To compensate for the fact that three-fold more $CD8^+Mac\text{-}1^-$ DC were used to obtain mRNA, the ligated cDNA was diluted by a factor of three. Aliquots (1 µl) of the ligation mixture were amplified in multiple 100 µl polymerase chain reactions (PCR) using R-Bgl-24. The PCR reaction contained; 66mM Tris-HCl (pH8.8), 4mM $MgCl_2$, 16mM $(NH_4)_2SO_4$; 33 µg/ml BSA, dATP, dCTP, dGTP, and dTTP (all 0.3 mM) and 2 µg R-Bgl-24 primer. The R-Bgl-12 oligonucleotide was melted away at 72°C (3 min) and the 3'ends were filled in with 5 U Taq DNA polymerase (Perkin Elmer) at 72°C (5 min). Twenty cycles of amplification were performed (1min, 95°C; 3 min, 72°C). Amplification products were visualised on a 1.3% agarose gel containing ethidium bromide which confirmed that each sample gave rise to a similar concentration of representations. Products of each representation were then combined, phenol extracted, ethanol precipitated and resuspended in TE at 0.5 µg/ml. The R-adapters were removed from the representation with *DpnII* and the digest was phenol extracted and ethanol precipitated to form the driver. Twenty micrograms of this driver was further gel-purified on a 1.2% TAE agarose gel, and the product, which was now free of the R-adapter, was isolated using QIAEX (Qiagen). This formed the "tester" of which 2 µg were ligated to the J-Bgl-12/24 adapter in the same manner as described above. For the first subtractive hybridisation step, 0.4 µg J-ligated tester ($CD8^+$ DC) was mixed with 40 µg of driver ($CD8^-$ DC) and *visa versa*. The mixture was phenol extracted, ethanol precipitated, and resuspended in 4 µl of EEx3 buffer (30 mM EPPS (Sigma), pH 8.0; 3 mM EDTA). The solution was

overlayed with mineral oil and the DNA was denatured for 6 min (98°C). The salt concentration was adjusted with 1 µl of 5M NaCl and the sample was allowed to anneal for 20 h (67°C). The hybridised sample was diluted with 8 µl TE (10mM Tris, 1mM EDTA, pH8.0) containing 5 mg/ml yeast RNA and then resuspended in a total volume of 400 µl TE. For each subtraction, four 200 µl PCR reactions containing 20 µl of hybridisation mix were set up as previously, but the primer was omitted. Again, the 12-mer oligo (R-Bgl-12) was melted away and 3' ends were filled using Taq DNA polymerase, then 2 µg of J-24-mer was added. After ten cycles of amplification, the four reactions were pooled, phenol extracted, isopropanol precipitated, and resuspended in 40 µl of 0.2xTE. Twenty microlitres of the product was digested with 20 U of mung bean nuclease and the reaction was stopped after 30 min by the addition of 50 mM Tris-HCl (pH8.9). The digest was heated to 98°C (5 min), chilled on ice then used in the final amplification. Four PCRs were conducted per hybridisation. Each PCR containing 20 µl of MBN-treated product and 2µg J-Bgl-24 was heated to 80°C, before 5 U of Taq DNA polymerase was added and further 18 amplification cycles were performed. The four reactions were pooled, phenol extracted, isopropanol precipitated, and resuspended at 0.5 µg/µl, giving the first differential product (DP1). The J-adapters were changed with N-Bgl-12/24 adapter and the process was repeated, with the exception that 50 ng tester was mixed with 40 µg of driver (i.e. 1:800). To generate the final DP3 product, 100 pg of J-ligated DP2 was mixed with 40 µg driver (i.e. 1:400,000) and the process was repeated except that the final amplification was performed for 22 cycles (70°C, 3 min; 95°C, 1 min).

3. Protein expression of FIRE domains

3.1. Oligonucleotides

Four cDNA constructs consisting of various extracellular domains of the FIRE clones were amplified by PCR using the following strategy:

(i) FIRE EGF domain 1;

Forward primer (5'-3'): CTAC GGATCC AAT ATT TCA GCT TCC TGT CC (SEQ ID NO:8);

Reverse primer (5'-3'): CGCG AAGCTT TCA ATC TTG AGA TTT GTC ATG G (SEQ ID NO:9).

(ii) FIRE EGF domain 2 Forward primer(5'-3'): GACG GGATCC AAT GAG TGT CTA CTG AAA GAA TTG (SEQ ID NO:10);
Reverse primer (5'-3'): ACCG AAGCTT TCA GCT CTT GTT CAC ATA ACA ATC (SEQ ID NO:11).

(iii) FIRE EGF domain 1 & 2;
Forward primer (5'-3'): CTAG GGATCC AAT ATT TCA GCT TCC TGT CC (SEQ ID NO:12);
Reverse primer (5'-3'): ACCG AAGCTT TCA GCT CTT GTT CAC ATA ACA ATC (SEQ ID NO:13).

(iv) FIRE Hinge;
Forward primer (5'-3'): ACAC GGATCC ACT TTG GGA GTA CTG AGT GAA (SEQ ID NO:14);
Reverse primer (5'-3'): CGCT AAGCTT TCA TAG AGC CAT GAG CAC AGC A (SEQ ID NO:15).

3.2. PCR Protocol

The oligonucleotide pairs listed above were used to amplify the corresponding FIRE domains from BlueScript plasmid DNA (1 µl of 1:10 dilution) containing FIRE cDNA inserts.

The final concentration of each oligonucleotide in the PCR was 400 nM, magnesium concentration was 2 mM and Elongase (Gibco-BRL) was used as the polymerase in all reactions. PCR were conducted over 32 cycles as follows (cycle 1 94°C, 1 min; cycle 2-31: 94°C, 30 sec, 58°C, 30 sec (or 55°C to amplify FIRE EGF1 domain), 68°C, 30 sec; cycle 32: 68°C, 5 min)

3.3. Cloning of PCR Products

PCR products were electrophoresed through 2% agarose gels containing ethidium bromide. The bands of interest were excised and the DNA was purified from the gel pieces using a 'Qiaex II Gel Extraction Kit' (Qiagen) according to the manufacturer's recommendations. The purified DNA from each PCR contained a *Bam*HI recognition site at its 5' end (indicated by the single underlined region in the sequences of all forward primers above). This *Bam*HI recognition site (and other restriction endonuclease recognition sites described below) was 'protected' during PCR by four non-specific flanking bases, indicated by double-underlining in Section 3.1. The DNA amplified by all primers of contructs (i)-(iv) (Section

3.1) contained a *Hind*III recognition sequence at its 3' end (indicated by single underline). All PCR products also contained a stop codon at their 3' ends (encoded in the reverse primer and shown in bold in section 3.1). Each of the purified PCR products was then cut with the appropriate restriction enzymes; ie; Constructs (i)-(iv) were cut with *Bam*HI and *Hind*III. Similarly, two vectors that had been chosen for bacterial expression, pMalp2 and pCaln, were treated with *Bam*HI and *Hind*III (both vectors). The PCR products were ligated into the appropriate vector and these plasmids were then used to transfect the *E.coli* strain, DH5α.

4. **Constructs and Immunisation Protocol**

FIRE and a control protein were expressed as FLAG tagged proteins on the surface of CHO cells. Briefly, primers (5' TAG TAG ACG CGT ATA TTA CAA ATG ATG AAT ATT (SEQ ID NO:20) and 5' TAG TAG ACG CGT TCA ATC ACT AAT AGT TCT GCT (SEQ ID NO:21)) were designed to amplify mouse FIRE without its leader sequence and to add adaptors that would allow subcloning into the pEF-BOS vector. The vector cDNA (http://www.wehi.edu.au/willson vectors) had been modified to contain the IL-3 leader sequence followed by the FLAG epitope and the cloning site that would allow the insertion of the FIRE cDNA. This construct resulted in the expression of FIRE proteins that contained the FLAG epitope at the N-terminus ie. extracellularly. Using FuGENE 6 Transfection Reagent (Boehringer Mannheim) CHO and 293T cells were co-transfected with the pEF-BOS-FIRE and a pCI-neo plasmid containing the neomycin phosphotransferase gene (kindly provided by Dr A Lew; Promega, Wisconsin) or pPGKpuroA (kindly provided by Leonie Gibson) at a ratio of 10:1. Transfectants were allowed to recover for 24 h before selection with 750 µg/ml G418 (Geneticin, GIBCO) commenced. FIRE-positive cells were stained with anti-FLAG mAb (IC7; kindly provide by Prof Nicola) followed by an anti-mouse-PE (Silenus) and isolated by sorting on MoFlow. After two rounds of this enrichment a pool of stable transfectants was established (Figure 1).

5. **Fc-Fusion proteins**

To produce soluble FIRE protein, the external portion of FIRE was amplified (using the following primers: 5' CGG GAT CCT CCT CAT GGG

GTA GAG CC (SEQ ID NO:22) and 5' CGG GTA CCA CCA TGG CAA GCA GGT GCC TTC TGC (SEQ ID NO:23)) then fused to the human IgG1 Fc domain and expressed in the Cigh vector (kindly provided by Dr A. Lew). The contruct was co-transfected with the pCI-neo plasmid into CHO cells. Transfectants were cloned by limiting dilution and clones that produced the Fc-fusion protein were selected using an anti-human Ig ELISA. Fc-FIRE was purified and enriched using an anti-human IgG agarose column (Sigma). The fusion protein was utilised in ELISA where an anti rat-HRP (Chemicon) antibody was used to detect sera that bound to Fc-FIRE.

6. Immunisations and Monoclonal Ab Production

Rats were immunised 4 times with 5-10 million CHO cells expressing FIRE-FLAG, then given a final boost four days prior to fusion. Hybridomas were produced by fusion of rat spleen cells with SP2/0 myeloma line using PEG 1500. Following HAT selection, wells containing hybridomas secreting specific monoclonal antibodies were identified by ELISA and FACS analysis of supernatants. Positive hybridomas were cloned by limiting dilution.

7. Immunohistological Analysis

Spleens were snap frozen in compound embedding medium (OCT) using liquid nitrogen. Sections (5 microns) were cut and fixed using ice-cold acetone. Fixed sections were first incubated with biotinylated FIRE mAb (3H7 and 6F12; 1 hr at room temperature), washed in PBS then incubated with ABC HRP kit (Vector Laboratories). The reaction was visualised using NovaRED (Vector Laboratories). Sections were counterstained with hematoxylin.

8. FACS Analysis

8.1 Splenic DC and Macrophages

Splenic DC were obtained as described previously (Vremec et al. 2000). To obtain splenic macrophages, spleens were mechanically disrupting by passing through a metal sieve. Cells were then resuspended in Nycodenz (1.091 g/cm^3) and the light density cells separated by density centrifugation. Irrelevant cells were then removed by incubation with anti-CD3 (KT3), anti-erythrocyte (TER119), and anti-B220 (RA36B2), anti-CD8 (53-6.7) anti-CD4

(GK1.5) followed by depletion using anti-rat Ig conjugated magnetic beads as per standard protocol (8). The macrophage enriched fraction was then stained with anti-M1/70 (CD11b) and anti-F4/80. Dead cells were excluded from analysis based on their uptake of propidium iodide (PI).

8.2 Peripheral Blood Mononuclear Cells

C57/B6 mice were bled by cardiac puncture into tubes containg heparin/PBS. Mononuclear cells were isolated by density centrifugation using lympholyte M (Cedarlane Laboratories). The light density cells were then incubated with mAb anti-CD3 (KT3), anti-Thy1.1 (T24/31.7), anti-Gr1 (RB68C5), anti-erythrocyte (TER119), and anti-B220 (RA36B2) and depleted using anti-Ig coupled Dynabeads (Vremec et al. 2000). The remaining mononuclear cells were stained and analysed for expression of FIRE.

9. Cloning of Human FIRE

The following 2 oligonucleotides 5' CACCTGCAGCTCTTCCATCT (SEQ ID NO:16) and 5' GAAAGTTTGCTTCTCAAAATCCA (SEQ ID NO:17), derived from sequences in the translated region of mouse FIRE, were used to amplify a fragment of human FIRE cDNA by low stringency PCR (annealing temperature: 50 degrees, Mg2+ concentration: 2.5mmol/l, 40 cycles) using target cDNA derived from both a human thymic preparation enriched in DCs and also from fresh and LPS activated human splenocytes. The resulting human FIRE sequence was 403 bp and had 83% homology with mouse FIRE at the DNA level. A 380 bp sub-fragment (isolated by PCR using the following primers 5' ggaagtagaacaccaggtttatca and 5' cctcttcctggcccacct) of the 403 bp human FIRE cDNA was then used to screen a commercial library (human bone marrow 5'-STRETCH cDNA library in lambda gt11, CLONTECH Laboratories, Palo Alto, CA) using conventional hybridisation methods. The resulting hybridising clone contained approximately 1482 bp of human FIRE. The remaining 5' human FIRE cDNA sequence was identified in clone RP11-1137G4 from the htgs database using a BLAST search. Primers 5' TGTCTCATTGCACCTCTTGGTTTCAT (SEQ ID NO:18) and 5' CCACAACAGCACCCACTGT (SEQ ID NO:19) were designed from sequences in clone RP11-1137G4 and used to amplify the 5' human FIRE cDNA using PCR.

RESULTS

1. Cloning of mouse FIRE

RDA analysis was performed to compare gene expression in the myeloid-related $CD8^-Mac\text{-}1^+$DC and the lymphoid-related $CD8^+Mac\text{-}1^-$DC. Results showed that as successive rounds of PCR and subtractive hybridisation occurred genes in common between the two populations were subtracted and not amplified (hence the background smear disappeared during progression from DP-1 (the first differential product) to DP-3 (the third differential product)). The bands which were observed in the DP-3 which corresponded to fragments of putative differentially expressed cDNA molecule (data not shown) were cloned and sequenced. House-keeping genes were detected only at very low frequency in these sequenced fragments suggesting that the RDA efficiently removed "common" sequences. To confirm that the bands from the DP-3 products were indeed differentially expressed in a minority population such as DC, several different approaches were taken. First, we generated a new "representation" from a separate source of RNA - this assures that any bias introduced in the first representation (used to generate the first RDA) would not be introduced in the reanalysis of differential expression. Using this approach, which is referred to as a "Virtual Northern", 9/11 gene fragments were found to be differentially expressed. This is a surprisingly high efficiency considering techniques such as RDA are prone to generating false positives, and indicates that this particular RDA successfully amplified differentially expressed genes. One of the DNA fragments was chosen for further analysis as it encoded a novel membrane protein, termed FIRE. Conventional Northern blot analysis confirmed data from Virtual Northerns and RT-PCR that FIRE is expressed at higher levels in myeloid DC than lymphoid DC.

Full length clones encoding this novel gene were obtained using conventional molecular biological techniques, involving the RDA generated fragments. The full length sequence of murine FIRE, showing both cDNA sequences and translated protein sequences, are shown in Figure 2.

FIRE encodes a 681 amino acid protein and analyses of the FIRE sequence shows that it is a novel member of the recently described EGF/TM7 superfamily (7), a comparison of the FIRE sequence with its distant relatives, the other members of the EGF/TM7 superfamily are shown in Figure 3. The

most famous member of the EGF/TM7 superfamily is F4/80 (mouse EMR-1) a molecule which is a marker of macrophages. Analysis of the FIRE sequence predicts a structure where there are two EGF domains in its extracellular region together with a "hinge" structure possessing a number of putative glycosylation sites. The protein then crosses the cell membrane seven times before a sizeable cytoplasmic domain of some 94 amino acids.

2. **Immunohistochemistry Analysis Results**

Four monoclonal antibodies (mAb) have been generated against mouse FIRE. Correspondingly, mAb could be generated against human FIRE.

Using the mAb against FIRE, it was determined that FIRE positive cells were predominantly present in the marginal zones and red pulp of mouse spleen. Such staining suggests that FIRE is expressed on DC present in the marginal zones and other antigen presenting cells (APC) such as macrophages that reside in the red-pulp.

3. **FACS Analysis Results**

In the mouse spleen, FIRE is predominantly expressed on the surface of DC (FIGURE 4A) and macrophages (FIGURE 4B and 4C). As suggested by the initial RDA results, FIRE is expressed more abundantly on the splenic DC that do not express CD4 and CD8, and to a lesser extent on the CD4+ DC and CD8+ DC (FIGURE 4A). Other cell types such as T and B cells do not appear to express FIRE, though it is possible that a very small population of either subsets express low levels of FIRE. A large proportion of blood mononuclear cells, including putative early DC, express high levels of FIRE (FIGURE 4E).

4. **Functional Data Results**

FIRE positive blood mononuclear cells could be the precursors of some CD8- lymphoid tissue DC. Peripheral blood mononuclear cells were isolated as described above. Cells were stained for FIRE expression, then sorted into population that were FIRE+ or FIRE- (MoFlow (Cytomation Inc., Fort Collins, CO). The sorted cells were incubated overnight at 37°C in medium alone or medium containing Fl3L, IL-4, GM-CSF, and TNF-α, then analysed for their expression of CD11c as a marker of DC. FIRE+ cells incubated in medium alone did not express high levels of CD11c (FIGURE 5A), but upon incubation with the cytokine cocktail, a large proportion of these cells

became CD11c+ (FIGURE 5B). Some CD11c+ cells were also generated from FIRE- cells, in medium alone (FIGURE5C) and medium plus cytokines (FIGURE 5D), though the proportion of these cells was not as high as when FIRE+ precursors were used. This data indicates that FIRE+ blood cells may be precursors of APC such as DC and macrophages.

5. Cloning and expression of human FIRE

The human FIRE DNA sequence was isolated as described in the materials and methods. Figure 6 shows the cDNA and amino acid sequences of human FIRE.

Human DC differentially express FIRE mRNA, where monocyte-derived DC express the highest level of FIRE whereas thymic DC express less message. Activation of DC via CD40 (using mAb against CD40) results in down-regulation of FIRE mRNA. Very little FIRE transcript could be detected by RT-PCR in T and B cells (see Table 1).

Table 1. The expression of human FIRE assessed by RT-PCR

Cell type	human FIRE expression
Thymic preparation enriched in DC	+
Total splenocytes	+ + +
Tonsillar $CD3^+$ T cells	-
Tonsillar $CD19^+$ B cells	-
Peripheral blood $CD14^+$ monocytes	+
Monocyte-derived DC	+ + +
CD40-activated Monocyte-derived DC	+
$CD11b^-$ thymic DC	-
CD40-activated $CD11b^-$ thymic DC	-
$CD11b^+$ thymic DC	+
CD40-activated $CD11b^+$ thymic DC	-

FIRE-specific primers used in RT-PCR were 5' ggaagtagaacaccaggtttatca (SEQ ID NO:24) and 5' cctcttcctggcccacct (SEQ ID NO:25).

FIRE is a marker for DC populations. The data obtained to date demonstrates that this molecule is expressed differentially or at much higher levels on the more "stimulatory" $CD8^-$ myeloid-related rather than the $CD8^+$ lymphoid-related dendritic cell populations, as they occur in the mouse spleen. Accordingly, it is believed that specific ligands such as monoclonal antibodies directed to this molecule will be useful reagents in identifying and particularly in purifying dendritic cells.

Publications referred to above are incorporated herein in their entirety by this reference.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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